PATENT
Attorney Docket No.: 19452A-000700US
Client Ref. No.: UCSD 99-100

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: LILJEGREN and YANOFSKY Application No.: 09/548,971 Filed: April 13, 2000 For: CONTROL OF FRUIT DEHISCENCE IN ARABIDOPSIS BY INDEHISCENT1 GENES	Examiner: Kruse, David H. Art Unit: 1638 DECLARATION UNDER 37 C.F.R. § 1.132 OF MARTIN F. YANOFSKY, PH.D.

Assistant Commissioner for Patents
Washington, D.C. 20231

I, Martin F. Yanofsky, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I graduated from the University of California at San Diego with a bachelor of science degree in biology in 1978. In 1986, I graduated from the University of Washington with a Ph.D. degree in microbiology. I have published more than fifty

scientific papers on the subject of plant biology. A copy of my curriculum vitae is attached hereto as Exhibit A.

3. I am currently a professor at the University of California, San Diego. I have been in this position for twelve years.

4. Two IND1 orthologs were isolated from *Brassica napus* plants. Since *Brassica napus* has an allotetraploid genome, it is not surprising that two different IND1 orthologs are present in the genome. The two sequences are designated Bn IND1 and Bn IND2. An alignment of the amino acid sequences of Bn IND1 and Bn IND2 with SEQ ID NO:2 are depicted in Exhibit A. The amino acid sequence of Bn Ind1 is approximately 79% identical to SEQ ID NO:2 of the present invention. The amino acid sequence of Bn Ind2 is approximately 74% identical to SEQ ID NO:2 of the present invention. Thus, both sequences are at least 70% identical to SEQ I DNO:2 of the present application.

5. Transformation of either Bn IND1 or Bn IND2 into *ind1* mutant *Arabidopsis* plants resulted in complementation of the mutant phenotype. These results demonstrate that Bn IND1 and Bn IND2 carry out the same basic functions as IND1.

6. These results provide two additional functional sequences within the scope of the present claims (i.e., polynucleotides encoding polypeptides at least 70% identical to SEQ ID NO:2). Thus there can be no question that gene products at least 70% identical to SEQ ID NO:2 do indeed function to control fruit dehiscence in plants.

Date: October 7, 2002

By: Martin F. Yanofsky
Martin F. Yanofsky, Ph.D.

Martin F. Yanofsky

Education

B.A.	1978	Biology	University of California at San Diego
Ph.D.	1986	Microbiology	University of Washington

Professional Experience

1990-1994	Assistant Professor, Department of Biology, University of California at San Diego, La Jolla, CA
1994-1998	Associate Professor, Department of Biology, University of California at San Diego, La Jolla, CA
1998-present	Professor, Department of Biology, University of California at at San Diego, La Jolla, CA

Awards and Honors

1985-1986	National Institutes of Health Biology Training Grant in Molecular and Cellular Biology
1986	Achievement Rewards for College Scientists (ARCS)
1987-1989	National Science Foundation Postdoctoral Fellowship in Plant Biology
1993-1994	Arnold and Mabel Beckman Young Investigator Award
1991-1995	David and Lucile Packard Fellowship for Science and Engineering

Editorial Board

1995-present	Developmental Biology
1999-present	Trends In Plant Science

Publications

Klee HJ, Yanofsky MF and Nester EW. (1985) Vectors for transformation of higher plants. **Bio/Technology** 3:637-642.

Yanofsky M, Montoya A, Knauf V, Lowe B, Gordon M and Nester E. (1985) Limited host range plasmid of *Agrobacterium tumefaciens*: Molecular and genetic analyses of transferred DNA. **J Bacteriol.** 163 No.1:341-348.

Yanofsky M, Lowe B, Montoya A, Rubin R, Krul W, Gordon M and Nester E. (1985) Molecular and genetic analyses of factors controlling host range in *Agrobacterium tumefaciens*. **Mol Gen Genet.** 201:237-246.

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Knauf V, Yanofsky M, Montoya A and Nester E. (1984) Physical and Functional Map of an *Agrobacterium tumefaciens* Tumor-Inducing Plasmid that Confers a Narrow Host Range. **J Bacteriol**. 160 No.2:564-568.

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Klee HJ, Yanofsky MF and Nester EW. (1985) Vectors for transformation of higher plants. **Bio/Technology** 3:637-642.

Yanofsky M, Montoya A, Knauf V, Lowe B, Gordon M and Nester E. (1985) Limited host range plasmid of *Agrobacterium tumefaciens*: Molecular and genetic analyses of transferred DNA. **J Bacteriol.** 163 No.1:341-348.

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Leroux B, Yanofsky MF, Winans SC, Ward JE, Ziegler SF and Nester EW. (1987) Characterization of the virA locus of *Agrobacterium tumefaciens*: a transcriptional regulator and host range determinant. **EMBO J.** 6 No.4:849-856.

Yamomoto A, Iwahashi M, Yanofsky MF, Nester EW, Takebe I and Machida Y. (1987) The promoter proximal region in the virD locus of *Agrobacterium tumefaciens* is necessary for the plant-inducible circularization of T-DNA. **Mol Gen Genet. 206**:174-177.

Ma D, Yanofsky MF, Gordon MP and Nester EW. (1987) Characterization of *Agrobacterium tumefaciens* strains isolated from grapevine tumors in China. **App Env Micro. 53** No.6:1338-1343.

Porter SG, Yanofsky MF and Nester EW. (1987) Molecular characterization of the virD operon from *Agrobacterium tumefaciens*. **Nucl Acids Res. 15** No.8:7503-7517.

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Bowman JL, Yanofsky MF and Meyerowitz EM. (1988) *Arabidopsis thaliana*: A review. Oxford Reviews of Plant Molecular Biology. ed. B.J. Miflin, Oxford Univ., New York 5:57-87.

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Mena, M., Mandel, M.A., Lerner, D., Yanofsky, M.F., and Schmidt, R.J. (1995). Characterization of the Maize MADS-Box gene family. **Plant J.** 8:845-854.

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Ferrándiz, C. Pelaz, S., and Yanofsky, M.F. (1999). Control of carpel and fruit development in Arabidopsis. **Annu. Rev. Biochem.** 68:321-354.

Sessions, A. and Yanofsky, M.F. (1999) Dorsoventral patterning in plants. **Genes and Development** 13:1051-1054.

Sessions, A., Weigel, D., and Yanofsky, M.F. (1999). The *Arabidopsis thaliana MERISTEM LAYER1* promoter specifies epidermal expression in meristems and young primordia. **The Plant J.** 20:259-263.

Ferrándiz, C., Gu,Q, Martienssen, R., and Yanofsky, M.F. (2000). Redundant Regulation of Meristem Identity and Plant Architechture by *FRUITFULL*, *APETALA1* and *CAULIFLOWER*. **Development**, 127:725-734.

Ng, M. and Yanofsky, M.F. (2000). Three ways to learn the ABCs. **Current Opp. Plant Biol.** 3:47-52.

Ambrose, B.A., Lerner, D.R., Ciceri P., Padilla, C.M., Yanofsky, M.F. and Schmidt, R.J. (2000). Molecular and genetic analyses of the *Silky1* gene reveals conservation in floral organ specification between eudicots and monocots. **Molecular Cell**, 5:569-579.

Weigel, D., J.H. Ahn, M.A. Blázquez, J.O. Borevitz, S.K. Christensen, C. Fankhauser, C. Ferrándiz, I. Kardailsky, E.J. Malancharuvil, M.M. Neff, J.T. Nguyen, S. Sato, Z. Wang, Y. Xia, R.A. Dixon, M.J. Harrison, C.J. Lamb, M.F. Yanofsky, and J. Chory. (2000) Activation Tagging in *Arabidopsis*. **Plant Physiol**,122:1003-1014 (2000).

Pelaz, S., G. Ditta, E. Baumann, E. Wisman and M. F. Yanofsky. (2000) B and C floral organ identity functions require *SEPALLATA* MADS-box genes. **Nature** 405: 200-203.

Alvarez-Buylla, E.R., S. Pelaz, S.J. Liljegren, S.E. Gold, C. Burgeff, G.S. Ditta L.R. de Pouplana, L. Martínez-Castilla, and M.F. Yanofsky. (2000). An ancestral MADS-box gene duplication occurred before the divergence of plants and animals. **Proc. Natl. Acad. Sci.** 97:5328-5333.

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Ferrándiz, C., Liljegrin, S.J., and M.F. Yanofsky. (2000). Negative regulation of the *SHATTERPROOF* genes by FRUITFULL during Arabidopsis fruit development. **Science** 289:436-438.

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PATENT
Attorney Docket No.: 19452A-000700US
Client Ref. No.: UCSD 99-100

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: LILJEGREN and YANOFSKY	Examiner: Kruse, David H.
Application No.: 09/548,971	Art Unit: 1638
Filed: April 13, 2000	DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. JOHAN BOTTERMAN, PH.D.
For: CONTROL OF FRUIT DEHISCENCE IN ARABIDOPSIS BY INDEHISCENT1 GENES	

Assistant Commissioner for Patents
Washington, D.C. 20231

I, Johan Botterman, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I graduated from University of Gent with a degree in chemistry and agriculture in 1981. In 1986, I graduated from University of Gent with a Ph.D.

degree in Agricultural Science. I have published more than forty scientific papers on the subject of plant biology. A copy of my curriculum vitae is attached hereto as Exhibit A.

3. I am currently a Research Manager at the Bayer CropScience in Belgium. I have been in this position for nine years.

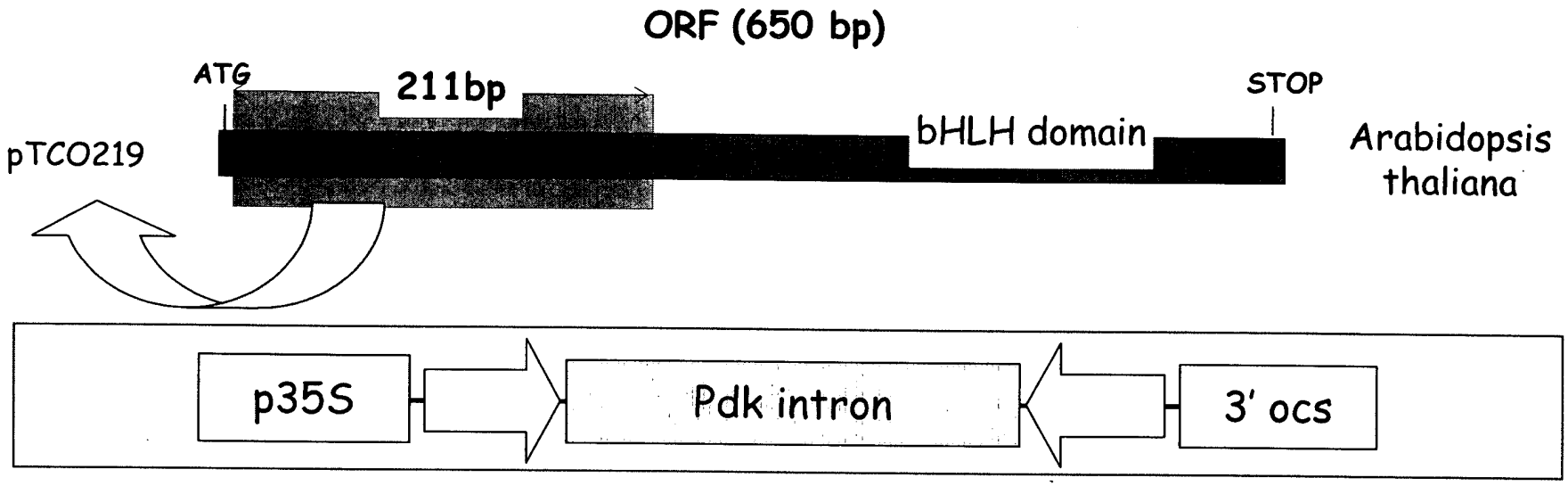
4. We have used an experimental approach based on RNA interference (RNAi) with fragments of the Bn IND1 and Bn IND2 genes from *Brassica napus* to downregulate the expression of the ind 1 gene in Arabidopsis. These experiments demonstrate that gene fragments about 65% identical to the corresponding *Arabidopsis IND1* fragments can be used to delay fruit dehiscence in transgenic Arabidopsis plants.

5. Specifically, restriction fragments from the 5' end of either Bn IND1 or Bn IND2 genes were cloned into vectors yielding the constructs pTCO212 (261 bp fragment) and pTCO218 (269 bp fragment), respectively. In addition, the first 211 base pairs of the *Arabidopsis IND1* sequence was inserted into the same vector to make construct pTCO219. *See*, Exhibit A. Each of these constructs result in the production of a transcript carrying an inverted repeat sequence of the respective gene fragments. The fragments from Bn IND1 and Bn IND2 have about 65% nucleotide sequence identity with *Arabidopsis IND1*. Transformation of either pTCO212, pTCO218, or pTCO219 into *Arabidopsis* resulted in plants with delayed fruit dehiscence. *See*, Exhibit B.

Date: 2/10/02

By: _____
Johan Botterman, Ph.D.

RNAi Strategy in Arabidopsis: IND1



	Sequence similarity to target	Transformants analysed	Podshatter Resistant
pTCO219	100%	203	199 (98%)

RNAi of IND1 in Arabidopsis

	Sequence similarity to target	Transformants analysed	Podshatter Resistant
pTCO212	~ 65%	152	50 (33%)
pTCO218	~ 65%	202	73 (36%)
pTCO219	100%	203	199 (98%)

CURRICULUM VITAE

Name : Johan H. Botterman
Address : Het Wijngaardeke 5
9840 Zevergem

Date of birth : 30-05-58

Education :

1981 University of Ghent - Faculty of Agriculture - engineer for chemistry and agricultural industries.

1981 - 1982 Grant from I.W.O.N.L.
1982 - 1986 Grant from N.F.W.O.
1982 - 1983 - European Molecular Biology laboratory - Heidelberg
1983 - 1986 - University of Ghent - Plant Genetic Systems

1986 University of Ghent - Faculty of Agriculture
Ph.D. in Agricultural Sciences

Working experience :

1986 - 1989 Plant Genetic Systems - Project leader Molecular Biology
Project : Engineering herbicide resistance in plants.
Responsibility for the molecular biology of the project : isolation and characterization of the bar gene; design of plasmid vectors for expression of the gene in E. coli and different plant species.

1989 - 1992 Plant Genetic Systems - Product Development Manager
Responsibilities : molecular biology group, cell and tissue culture group.
Responsibility for the molecular biology and cell biology units as service units for different crops. The molecular biology involved 1) the isolation and characterization of genes as potential traits in crop species (insect control, pollination control, bacteria resistance; improvement of digestibility in forage crops); 2) design of vectors for transformation of different crop species; 3) molecular characterization of transgenic lines.
The cell biology involved different groups focused on the transformation/regeneration of dicotyledonous and monocotyledonous crop species. Transformation projects have been performed in tobacco, tomato, potato, oilseed rape, cabbages, alfalfa, poplar and maize.

1993 - 1996 Plant Genetic Systems - Research Manager

1997 - 1998 Plant Genetic Systems - Head of Biotechnology Research - Brassica oilseeds and vegetables.
Responsibilities :

- management of the research projects in oilseed rape towards the development of new added value traits in oilseed rape. Activities are focused on experimental approaches towards crop protection and crop improvement.

- management of research projects in vegetables conducted as service within the frame of collaborations with third parties
- management of groups focused on the development of molecular tools for breeding : molecular breeding and PCR diagnostics.

a. Publications :

1.
Effects of overproduction of tobacco MnSOD in maize chloroplasts on foliar tolerance to cold and oxidative stress
Frank Van Breusegem, Luit Slooten, Jean-Marie Stassart, Johan Botterman, Tanya Moens, Marc Van Montagu and Dirk Inzé
Journal of Experimental Botany, Vol. 50, No. 330, pp. 000, January 1998

2.
The use of Agrobacterium for Plant Genetic Engineering
Kathleen D'Halluin and Johan Botterman
p 339 - 344

3.
Processing of a chimeric protein in chloroplasts is different in transgenic maize and tobacco plants
Frank Van Breusegem, Sergei Kushnir, Luit Slooten, Guy Bauw, Johan Botterman, Marc Van Montagu and Dirk Inzé
Plant Molecular Biology 38: 491-496

4.
Field Testing of Insect and Herbicide Resistant Crops
J. Botterman and J. Leemans
Vortr. Pflanzenzüchtg. 16, 455-461 (1989)

5.
Engineering of Herbicide Resistance in Plants
J. Botterman and J. Leemans
Biotechnology and Genetic Engineering Reviews - Vol. 6, September 1988, p 321-340 (1988)

6.
Improvement of agricultural crops by genetic engineering
J. Botterman
Med. Fac. Landbouww. Rijksuniv. Gent, 53(4a), p 1695-1699 (1988)

7.
Engineering herbicide resistance in plants by expression of a detoxifying enzyme
M. De Block, J. Botterman, M. Vandewiele, J. Dockx, C. Thoen, V. Gosselé, N. Rao Movva, C. Thompson, M. Van Montagu and J. Leemans
The EMBO Journal vol. 6 no. 9 pp 2513-2518, 1987

8.
Evaluation of herbicide resistance in transgenic crops under field conditions
Willy De Greef, René Delon, Marc De Block, Jan Leemans and Johan Botterman
Bio/Technology Vol. 7, January 1989, p 61-64 (1989)

9.
Engineering herbicide resistance in plants
J. Botterman and J. Leemans
TIG - August 1988, Vol. 4, no. 8, p 219-222 (1988)

10.
Characterization of the herbicide-resistance gene bar from Streptomyces hygroscopicus
Charles J. Thompson, N. Rao Movva, Richard Tizard, Reto Crameri, Julian E. Davies, Marc Lauwereys and Johan Botterman
The EMBO Journal Vol. 6 No. 9 pp. 2519-2523, 1987

11.
Characterizaton of phosphinothricin acetyltransferase and C-terminal enzymatically active fusion proteins
Johan Botterman, Veronique Gosselé, Chris Thoen and Mark Lauwereys
Gene, 102 (1991) 33-37

12.
Plant and mammalian sorting signals for protein retention in the endoplasmic reticulum contain a conserved epitope
Jürgen Denecke, Riet De Rycke and Johan Botterman
The EMBO Journal vol. 11, no. 6 pp. 2345-2355, (1992)

13.
The Tobacco Luminal Binding Protein is Encoded by a Multigene Family
Jürgen Denecke, Maria Helena S. Goldman, Jan Demolder, Jef Seurinck and Johan Botterman
The Plant Cell, Vol. 3, 1025-1035, September (1991)

14.
Inhibition of Fungal Disease Development in Plants by Engineering Controlled Cell Death
Günter Strittmatter, Jan Janssens, Chris Opsomer and Johan Botterman
Bio/Technology, Vol. 13, p 1085-1089, October 1995

15.
Transformation of sugarbeet (Beta Vulgaris L.) and evaluation of herbicide resistance in transgenic plants
Kathleen D'Halluin, Martien Bossut, Els Bonne, Barbara Mazur, Jan Leemans and Johan Botterman
Bio/Technology Vol. 10, p 309-314, March 1992

16.
Plant molecular farming: production of peptides and proteins with high-added value
Johan Botterman and Enno Krebbers
Med. Fac. Landbouww. Rijksuniv. Gent, 54 (4a), 1989

17.
Expression of a bacterial lysine decarboxylase gene and transport of the protein into chloroplasts of transgenic tobacco
S. Herminghaus, P.H. Schreier, J.E.G. Mc.Carthy, J. Landsmann, J. Botterman and J. Berlin
Plant Molecular Biology 17: 475-486, 1991

18.
Engineering of Herbicide-Resistant Alfalfa and Evaluation under Field Conditions
Kathleen D'Halluin, Johan Botterman and Willy De Greef
Crop Science 30:866-871 (1990)

19.
Hybrid genes in the analysis of transformation conditions: II. Transient expression vs stable transformation - analysis of parameters influencing gene expression levels and transformation efficiency.
I. Negrutiu, J. Dewulf, M. Pietrzak, J. Botterman, E. Rietveld, E.M. Wurzer-Figurelli, De Ye and M. Jacobs
Physiologia PLANTARUM 79:197-205. Copenhagen 1990

20.
Expression of Dicistronic Transcriptional Units in Transgenic Tobacco
G. Angenon, J. Uotila, S.A. Kurkela, T.H. Teeri, J. Botterman, M. Van Montagu and A. Depicker
Molecular and Cellular Biology, Dec. 1989 p. 5676-5684

21.
Enkephalins produced in transgenic plants using modified 2S seed storage proteins
Joel Vandekerckhove, Jozef Van Damme, Mieke Van Lijsebettens, Johan Botterman, Marc De Block, Martine Vandewiele, Ann De Clercq, Jan Leemans, Marc Van Montagu and Enno Krebbers
Bio/Technology Vol. 7, September 1989, p 929-932

22.
Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants
Chris Bowler, Luit Slooten, Saskia Vandenbranden, Riet De Rycke, Johan Botterman, Chris Sybesma, Marc Van Montagu and Dirk Inzé
The EMBO Journal, vol. 10, no. 7 pp1723-1732, 1991

23.
Expression of a bacterial lysine decarboxylase gene and transport of the protein into chloroplasts of transgenic tobacco
S. Herminghaus, P.H. Schreier, J.E.G. McCarthy, J. Landsmann, J. Botterman and J. Berlin
Plant Molecular Biology 17: 475-486, 1991

24.
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D.E. de Oliveira, L.O. Franco, C. Simoens, J. Seurinck, J. Coppieters, J. Botterman and M. Van Montagu
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